

**Application
for
United States Letters Patent**

To all whom it may concern:

Be it known that I, Riccardo Dalla-Favera

have invented certain new and useful improvements in

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

of which the following is a full, clear and exact description.

IDENTIFICATION OF GENES ALTERED IN MULTIPLE MYELOMA

The invention disclosed herein was made with Government support under NIH Grant No. CA 44025. Accordingly, the U.S. Government has certain rights in this invention.

Background of the Invention

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found at the end of this application, preceding the claims.

Multiple myeloma (MM) is an incurable B cell tumor affecting B cell end-stage differentiation. Clinically, the course of MM is similar to end-stage plasma cell leukemia (PCL), i.e., there is an uncontrollable proliferation of myeloma cells accompanied by numerous complications, including hyperviscosity syndromes, hypercalcemia, infections, multiple bone fractures, and organ failure.

Non-random chromosomal translocation is known to play a crucial role in the tumorigenesis of hematologic malignancies (1). In B-cell lymphomas, many important proto-oncogenes deregulated by juxtaposition to immunoglobulin (Ig) gene locus have been identified. Each proto-oncogene is associated with a specific subtype of lymphoma, such as c-MYC in Burkitt's lymphoma, Cyclin D1 in mantle cell lymphoma, BCL-2 in follicular lymphoma and BCL-6 in diffuse large cell lymphoma (2-8). In contrast, little is known about molecular alterations of

human MM/PCL, due to the difficulty in cytogenetic analysis. However, previous cytogenetic reports have shown a 14q+ chromosome, suggesting the existence of a chromosomal translocation involving the Ig heavy chain (IgH) locus, is
5 observed in 20 ~ 30 % of the MM/PCL cases and it is the most frequent consistent abnormality (9-12). Even in such cases, most cytogenetic data have failed to identify donor chromosomes other than 11q13, 8q24, and 18q21, where proto-oncogenes Cyclin D1, BCL-2, c-MYC and BCL-2 are
10 located, respectively. Among them, the 11q13 locus has been demonstrated to be involved in nearly 5~10% of the cases and also in 62% of the established cell lines (13). The t(11;14)(q13;q32) translocation is also accompanied by a corresponding overexpression of the Cyclin D1 gene, which
15 raises a strong possibility of the involvement of this gene, although the breakpoints at 11q13 do not cluster like those of the lymphoma cases (14-16). Recent advances in fluorescence in situ hybridization (FISH) have made it possible to clarify both the frequency of the 14q+ chromosomes and the partner chromosomes of the IgH loci.
20 One such report revealed an intriguing result, i.e., that numerous chromosomal loci are able to translocate to IgH locus, including 6p21, 1q21, 3p11, 7q11, 11q23 (17). This has prompted a search for the proto-oncogenes deregulated by the regulatory elements of the IgH gene for a further
25 understanding of the molecular mechanisms of MM/PCL. In the present study, one candidate proto-oncogene, MUM1 (multiple myeloma oncogene 1), was found juxtaposed to the IgH gene as a result of t(6;14)(p25; q32) translocation in human myeloma cell line, SKMM-1. Over expression of the MUM1 mRNA was
30 observed in this cell line. A second gene, called MUM-2 was found translocated in proximity to the IgH gene on

chromosome 14q32 in human myeloma cell line, U-266.

5 The method of analysis of 14q+ chromosomal translocations
and identification of the genes altered in multiple myeloma
of this invention are useful since 1) no method is currently
available to determine the chromosomal sequences involved in
14q+ translocations, the most important cytogenetic lesions
associated with MM pathogenesis; 2) no specific gene lesion
is currently known for MM; 3) no diagnostic method based on
10 gene/DNA lesion is currently available for MM and 4) there
are no therapeutic approaches aimed at counteracting the
action of abnormal gene products in MM.

Summary of the Invention

This invention provides a method of determining a chromosomal breakpoint in a subject suffering from multiple myeloma which comprises steps of: (a) obtaining a DNA sample from the subject suffering from multiple myeloma; (b) determining whether there is J and C disjunction in the immunoglobulin heavy chain gene in the obtained DNA sample; (c) obtaining a genomic library having clones which contain genomic DNA fragments from the DNA sample which shows positive J and C disjunction; (d) selecting and isolating clones of the obtained library which show positive hybridization with a probe which is capable of specifically hybridizing with the C but not the J region of the immunoglobulin heavy chain gene; (e) preparing fluorescent probes from the genomic DNA fragments of the isolated clones from step (d); (f) hybridizing said fluorescent probes with metaphase chromosomes; and (g) determining the identity of the chromosomes which are capable of hybridizing to said fluorescent probes, wherein the identification of a chromosome other than chromosome 14 would indicate that the chromosomal breakpoint is between chromosome 14 and the identified chromosome, thereby determining a chromosomal breakpoint in a subject suffering from multiple myeloma.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than

the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from step (b); d) determining the sequence of the mRNA in the isolated complex, thereby determining the identity of the gene.

10 This invention provides a gene designated *MUM-1*. This invention provides a gene designated *MUM-2*. This invention provides an isolated nucleic acid molecule encoding a MUM protein. This invention provides a DNA encoding a MUM protein. This invention provides a cDNA encoding a MUM protein. This invention provides a genomic DNA molecule encoding a MUM protein. This invention provides a RNA molecule encoding a MUM protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-1 protein. This invention provides an isolated nucleic acid molecule encoding a human MUM-2 protein. This invention provides an isolated nucleic acid molecule encoding a MUM protein operatively linked to a promoter of RNA transcription. This invention provides a vector comprising the an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein. This invention provides a vector which comprises an isolated cDNA encoding a MUM protein, wherein the vector is a plasmid. This invention provides a host cell for the vector which comprises an isolated cDNA encoding a MUM protein.

This invention provides a nucleic acid probe comprising a

nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a MUM protein. This invention provides a nucleic acid probe
5 comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein.

This invention provides a nucleic acid probe comprising a
10 nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

This invention provides a nucleic acid probe comprising a
15 the sequence of a nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

This invention provides a nucleic acid probe comprising a
20 the sequence of a nucleic acid molecule encoding a MUM-2 protein which is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14.

This invention provides a method for detecting a
25 predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1
30 protein. This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the

subject a rearrangement of nucleic acid encoding MUM-2 protein.

5 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule
10 encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

15 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an isolated cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the isolated genomic DNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence
20 capable of specifically hybridizing to an isolated RNA molecule encoding a MUM protein.

25 This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically recognizing MUM-1 protein. This invention provides a purified MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention
30 provides an antibody capable of specifically recognizing a MUM-2 protein.

This invention provides a pharmaceutical composition

comprising an amount of an oligonucleotide effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane. This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

Brief Description of the Figures

Figure 1. JH-C μ dissociation in *Bam*HI digested DNA of the 14q+ SK-MM-1 cell line. A 10 μ g of the high molecular weight DNA was completely digested with *Bam*HI, loaded on each lane and blotted. The same filter was sequentially hybridized with JH, C μ , Cy2, and 0.7B/H probes. JH probe detects two rearranged bands of 12.0 kb and 9.7kb. The 9.7 kb band is comigrated with that probed with Cy2 probe, suggesting it to be a physiological rearrangement. On the other hand, one allele of the C μ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Therefore, 12.0 kb and 6.5 kb bands detected by JH and C μ (shown by arrowheads) might represent unknown derivative chromosome and derivative 14 chromosome, respectively. As expected, 0.7B/H probe (Fig. 2A) detected the rearranged band comigrated with 6.5kb band of C μ . Dashed lines show the comigration. Size markers of λ /*Hind*III are shown on the left.

Figures 2A-B. Molecular cloning of the breakpoints of the t(6;14) translocation and germline walking at MUM1 locus. (A) Restriction maps of λ SKB-4a and λ SKS-3 clones representing derivative 6 and 14 are shown, together with germline maps of IgH locus at 14q32 and MUM1

locus at 6p25. Arrows indicate the chromosomal breakpoints. B, *Bam*HI; E, *Eco*RI; H, *Hind*III. (B) Comparison of the nucleotide sequences around the breakpoints on derivative 6 and derivative 14 chromosome. Homologous regions are indicated by dashes. The arrow indicates the breakpoint. Nucleotide numbers shown below are the same as in the *S_μ* sequence reported by Sun, et al. (18).

Figure 3. Mapping of the MUM1 locus to chromosome 6p25. λ MUM-3 genomic clone (Figure 2A) was used as a probe for *in situ* hybridization. The white arrow indicates the fluorescence signal on chromosome 6 band p25. Right panel shows the G-banding picture stained with DAPI.

Figures 4A-C. Expression of the MUM1 gene in hematopoietic lineage. A 10 μ g aliquot of total RNA was loaded on each lane and Northern blot analysis was performed using the 2.1H probe (Figure 2A). GAPDH or β -actin probes were used to control for amount of RNA loaded. (A) MUM1 RNA expression in various hematopoietic cell lines. MUM1 RNA is detected in B cell and mature T cell lines as a single 6kb transcript. HELA, epithelial lineage; LCL, Epstein-Barr virus-transformed lymphoblastoid cell line; RAMOS and SK-MM-1, B-cell lineage; HUT-78 and MOLT-4, T-cell

lineage; HL-60 and U937, myelomonocytic lineage; K562, erythroid lineage. Dashes indicate 28S and 18S. (B) Expression in B cell lines derived from various stages of B cell differentiation. MUM1 RNA is seen throughout the B cell development except for BJAB cell line. 697, pre-B cell stage; RAMOS and BJA-B, Burkitt cell line representing mature-B cell stage; RPMI-8226 and U-266, plasma cell stage. (C) Comparison of the expression level among myeloma cell lines. MUM1 RNA is overexpressed in SK-MM-1 cell line carrying t(6;14). Overexpression of the MUM1 is also demonstrated in XG-4, XG-7, and XG-10 cell lines. RPMI-8226, U-266, EJM, and SKMM-1 are IL-6 (interleukin-6) independent lines, whereas XG-1, XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10 are IL-6 dependent lines.

Figures 5A-B. Sequence of MUM1 cDNA and structure of its predicted protein product. (A) Restriction map of the MUM1 cDNA and the position of the open reading frame (box). The solid box indicates approximate position of the DNA binding domain. Sc, *SacII*; A, *ApaI*; P, *PstI*; H, *HindIII*; S, *SacI* (B) Nucleotide sequence of the MUM1 cDNA and corresponding amino acid sequence. Putative translation initiation codons and preceding stop codons appearing in frame are underlined. The asterisk indicates the translation stop

codon.

Figures 6A-B. Homology between MUM1 and other IRF family proteins. (A) Similarity at N-terminal DNA binding domain. Black background indicates identical residues found more than four times. Gray indicates conserved residues that appear in at least four sequences at a given position. --- Conserved tryptophan residues in DNA binding domain among IRF family members are indicated by closed circles. (B) Similarity at C-terminal region between human MUM1, Mouse LSIRF/Pip, Human ICSBP, Human ISGF3γ, and Human IRF-3. Black and gray background are as in (A).

Figure 7. Genomic organization of the MUM1 gene and location of the chromosomal breakpoints in multiple myeloma. Filled boxes indicate the coding regions and empty boxes indicate the noncoding regions. The position, and the size of each exon of the MUM1 gene are approximate and have been determined by the hybridizations. One exon in each restriction fragment may consist of more than two exons. Translation initiation codon (ATG) and stop codon (TGA) are indicated. Genomic probes used for further investigations are shown as solid bars below the map. Arrows indicate the chromosomal breakpoints of SKMM-1 cell line and case 10. B, *Bam*HI; E, *Eco*RI; H, *Hind*III.

Figure 8. Scheme of the t(6;14)(p25;q32) translocation involving the MUM1 and the immunoglobulin heavy chain (IgH) gene loci. VH-D-J-CH indicates variable-diversity joining-constant region of the IgH gene. Direction of the MUM1 gene on the chromosome 6 is tentatively drawn.

Figure 9A-B. Demonstration of JH-C α disjunction in U-266 cells and cloning of normal and 14q+ chromosomal breakpoints. (A) The panel shows the results of Southern blot analysis of BamHI digested U-266 and normal control (placenta) genomic DNA using the indicated JH and C α probes. The arrowheads indicate two DNA fragments containing C α sequences not linked to JH sequences, suggesting the presence of a chromosomal breakpoint in 14q32. (B) The panel provides a schematic representation of the phage clones isolated from a library constructed from U-266 DNA and screened with a C α probe. Based on restriction enzyme analysis, the three cloned regions represent a normal C α region (14q32 germ-line), and two rearranged regions (der.14 and 14q32) containing unknown sequences linked to C α sequences. The 2.5BE probe used for Northern blot analysis of MUM2 transcripts (Fig. 10) is also shown.

Figure 10. Identification of MUM2 RNA transcripts. The

figure shows the results of a Northern blot analysis of RNA extracted from various MM/PCL cell lines using the 2.5BE probe (see Fig. 9) or GAPDH probe (as a control for RNA loading). A 1.9 Kb RNA transcript is detectable in some cell lines including U-266, indicating that the 2.5BE fragments represents part of a gene, MUM2.

Figure 11A-B. Schematic representation of IgH DNA rearrangements in normal B cells and in tumors carrying chromosomal translocations breaking the S region of the IgH locus. Note that in physiological IgH rearrangements (panel 11A) JH sequences and C sequences (C_μ before and C_γ after switch recombination, respectively) are consistently found within the same BamHI restriction fragment. Conversely, JH and C sequences are not linked, and are present on two different chromosomes [derivative X and derivative 14(14q+)] in cells carrying a chromosomal translocation breaking the switch region (panel 11B)

Figure 12A-B. MUM1 cDNA. cDNA insert is cloned into EcoRI/BamHI site of the pBluescript KS+. Bacteria strain used is DH5α cells. pcMUM1.16a contains full length open reading frame of nt. 217-1572.

Figure 13. Breakpoint Cloning of the U-266 Cell Line.

-15-

pMUM2-8 has a 22.0 KB insert in BamHI site
of pBluescript KS+.

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CONFIDENTIAL

Detailed Description of the Invention

The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

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C=cytosine A=adenosine
T=thymidine G=guanosine

10 This invention provides a method of determining a
chromosomal breakpoint in a subject suffering from multiple
myeloma which comprises steps of: (a) obtaining a DNA sample
from the subject suffering from multiple myeloma; (b)
determining whether there is J and C disjunction in the
immunoglobulin heavy chain gene in the obtained DNA sample;
15 (c) obtaining a genomic library having clones which contain
genomic DNA fragments from the DNA sample which shows
positive J and C disjunction; (d) selecting and isolating
clones of the obtained library which show positive
hybridization with a probe which is capable of specifically
20 hybridizing with the C but not the J region of the
immunoglobulin heavy chain gene; (e) preparing fluorescent
probes from the genomic DNA fragments of the isolated clones
from step (d); (f) hybridizing said fluorescent probes with
metaphase chromosomes; and (g) determining the identity of
25 the chromosomes which are capable of hybridizing to said
fluorescent probes, wherein the identification of a
chromosome other than chromosome 14 would indicate that the
chromosomal breakpoint is between chromosome 14 and the
identified chromosome, thereby determining a chromosomal
30 breakpoint in a subject suffering from multiple myeloma.

In an embodiment, step (b) of the above described method of
this invention is performed by Southern blotting. In
another embodiment, step (b) of the above method of this

invention is performed by polymerase chain reaction (PCR) with appropriate probes. Polymerase chain reaction is well known in the art. Since the sequences of both the C and J regions of an immunoglobulin heavy chain gene are known,
5 appropriate probes for PCR may routinely be designed.

In an embodiment, the genomic library is a phage vector library. In another embodiment, the genomic DNA fragments are generated by cleaving genomic DNA from cells of the
10 subject with an appropriate restriction enzyme. In a further embodiment, the restriction enzyme is *Bam*HI. In an embodiment, the restriction enzyme is *Sau*3AI. In another embodiment, the probe of step (d) is a human IgH J region JH probe. In a further embodiment, the probe of step (d) is a
15 human IgH C μ probe. In an embodiment, the probe of step (d) is a human IgH C γ 2 probe. In another embodiment, the chromosomal breakpoint identified is a t(6;14)(p25;q32) translocation. In an embodiment, the chromosomal breakpoint identified is a t(14;15) translocation.

This invention provides a method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma which comprises
20 steps of: a) selecting a probe having a sequence of a chromosome other than chromosome 14, identified at the chromosomal breakpoint detected in a subject suffering from multiple myeloma, wherein said probe is capable of hybridizing to the unique sequence of the gene other than
25 the immunoglobulin gene altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma; b) contacting said probe with mRNA isolated from a cell under conditions permitting formation of a complex between said probe and the mRNA; c) isolating the complex resulting from
30 step (b); and d) determining the sequence of the mRNA in the
35

isolated complex, thereby determining the identity of the gene.

5 In an embodiment, step (d) of the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma comprises steps of: i) synthesizing complementary DNA to the mRNA; and ii) performing sequence analysis of the complementary DNA to
10 determine the sequence of the mRNA.

15 This invention provides a gene identified by the method to identify a gene other than the immunoglobulin gene which is located in chromosome 14, altered by a chromosomal breakpoint detected in a subject suffering from multiple myeloma.

20 As used herein, "MUM" means any gene rearranged in 14q+ chromosomal abnormalities associated with multiple myeloma.

25 This invention provides a gene identified by the above method designated *MUM-1*. This invention provides a gene identified by the above method designated *MUM-2*.

30 This invention provides a gene identified by the above method, wherein the gene identified comprises a nucleic acid encoding a MUM protein. In an embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-1 protein. In another embodiment, the gene identified by the above method comprises a nucleic acid encoding a MUM-2 protein.

This invention provides an isolated nucleic acid molecule encoding a MUM protein. In an embodiment, the isolated

nucleic acid molecule encoding a MUM protein is a DNA molecule. In another embodiment, the isolated nucleic acid molecule encoding a MUM protein is a cDNA molecule.

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SUB B2
10 In an embodiment, a cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pcMUM1-1.6a (ATCC Accession No.). Plasmid pcMUM1-1.6a was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pcMUM1-1.6a
15 was accorded ATCC Accession Number _____.

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SUB B3
In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-2.4B/N (ATCC Accession No.). Plasmid pMUM1-2.4B/N was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of
25 Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-2.4B/N was accorded ATCC Accession Number _____.

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SUB B4
In another embodiment, a partial cDNA nucleic acid molecule encoding a MUM-1 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM1-7.7B (ATCC Accession No.). Plasmid pMUM1-7.7B was deposited on May 28, 1996 with the American Type Culture Collection

SUB B4
CONT

(ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM1-7.7B was accorded ATCC Accession Number _____.

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SUB B5

In another embodiment, a partial cDNA of the nucleic acid molecule encoding a MUM-2 protein is cloned into a pBluescript KS+ and the resulting plasmid is designated as pMUM2-8 (ATCC Accession No. _____). Plasmid pMUM2-8 was deposited on May 28, 1996 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Plasmid pMUM2-8 was accorded ATCC Accession Number _____.

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SUB B6

In an embodiment, the isolated DNA molecule encoding a MUM protein is a cDNA molecule having the nucleotide sequence shown in Figure 5B (SEQ. ID NO _____).

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In an embodiment, the isolated DNA molecule encoding a MUM protein is genomic DNA molecule. In an embodiment, the isolated nucleic acid molecule encoding a MUM protein is an RNA molecule.

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In an embodiment, the isolated nucleic acid encodes a human MUM-1 protein. In another embodiment, the isolated nucleic acid molecule encodes a human MUM-2 protein.

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SUB B7

In an embodiment, isolated nucleic acid molecule encodes the a human MUM-1 protein having substantially the same amino acid

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isolated nucleic acid molecule encoding a MUM protein.

As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-1. In an embodiment, the nucleic acid probe specifically hybridizes with nucleic acid encoding MUM-2. In an embodiment, the nucleic acid probe is complementary to nucleic acid encoding MUM-2.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a RNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a RNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is a genomic DNA probe. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is a genomic DNA probe.

In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is labeled with

a detectable marker. In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-2 is labeled with a detectable marker.

- 5 In an embodiment, the detectable marker is selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

10 In an embodiment, the nucleic acid probe which specifically hybridizes with nucleic acid encoding MUM-1 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14. In an embodiment, the nucleic acid probe which specifically
15 hybridizes with nucleic acid encoding MUM-2 is linked to a nucleic acid sequence capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule of human chromosome 14.

20 This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid
25 molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid
30 molecule encoding a MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-2 protein which is linked to a
5 nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic
10 acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding a MUM-1 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of
15 human chromosome 14.

In an embodiment, the nucleic acid probe comprises a nucleic
acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid
20 molecule encoding a MUM-2 protein which is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 14.

In an embodiment, the specific break point of the nucleic
25 acid probe comprises a portion of the t(6;14)(p25;q32) translocation. In an embodiment, the specific break point of the nucleic acid probe comprises a portion of a t(14;15) translocation. In an embodiment, the nucleic acid probe
30 comprising a portion of the t(6;14)(p25;q32) translocation is labeled with a detectable marker. In an embodiment, the nucleic acid probe comprising a portion of a t(14;15) translocation is labeled with a detectable marker. In an

embodiment, the nucleic acid probe comprising a portion of the t(6;14)(p25;q32) or comprising a portion of a t(14;15) translocation of claim 60, has a detectable marker selected from the group consisting of a radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein.

This invention provides a method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by contacting the nucleic acid from the sample with a MUM-1 probe under conditions permitting the MUM-1 probe to hybridize with the nucleic acid encoding MUM-1 protein from the sample, thereby detecting the rearrangement of nucleic acid encoding MUM-1 protein in the sample.

In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by contacting the nucleic acid from the sample with a MUM-2 probe under conditions permitting the MUM-2 probe to hybridize with the nucleic acid encoding MUM-2 protein from the sample, thereby

detecting the rearrangement of nucleic acid encoding MUM-2 protein in the sample.

5 In an embodiment, the rearrangement of nucleic acid encoding MUM-1 protein is detected by a MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid
10 molecule of human chromosome 14.

15 In an embodiment, the rearrangement of nucleic acid encoding MUM-2 protein is detected by a the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein which is linked to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

20 In an embodiment, the MUM-1 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-1 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a
25 nucleic acid molecule of human chromosome 14.

30 In an embodiment, the MUM-2 probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to a sequence of the isolated nucleic acid molecule encoding MUM-2 protein is linked at a specific break point to a nucleic acid sequence complementary to a sequence of a nucleic acid molecule of human chromosome 15.

35 In an embodiment, the MUM-1 probe comprises a specific break point comprising a portion of the t(6;14)(p25;q32)

translocation. In an embodiment, the MUM-2 probe comprises a specific break point comprising a portion of a t(14;15) translocation.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns

are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

5 In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein
10 comprises: a) obtaining DNA from the sample of the subject suffering from multiple myeloma; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) separating the resulting DNA fragments by size fractionation; d) contacting the resulting DNA fragments
15 with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point
20 to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; e) detecting labeled bands which have hybridized to the nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule
25 encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 to create a unique band pattern specific to the DNA of subjects suffering from multiple
30 myeloma; f) preparing DNA obtained from a sample of a subject for diagnosis by steps (a-e); and g) comparing the detected band pattern specific to the DNA obtained from a sample of subjects suffering from multiple myeloma from step (e) and the DNA obtained from a sample of the subject for

diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

5

In an embodiment, the size fractionation in step (c) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope, enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-1 protein comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a human MUM-1 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-1 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 14 and labeled with a detectable marker; d) detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from

step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple myeloma if the patterns are the same.

5 In an embodiment, the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-2 protein in a sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein
10 comprises: a) obtaining RNA from the sample of the subject suffering from multiple myeloma; b) separating the RNA sample by size fractionation; c) contacting the resulting RNA species with a nucleic acid probe capable of specifically hybridizing with a unique sequence included
15 within the sequence of a nucleic acid molecule encoding a human MUM-2 protein, wherein the sequence of a nucleic acid molecule encoding a MUM-2 protein is linked at a specific break point to a specified nucleic acid sequence of human chromosome 15 and labeled with a detectable marker; d)
20 detecting labeled bands which have hybridized to the RNA species to create a unique band pattern specific to the RNA of subjects suffering from multiple myeloma; e) preparing RNA obtained from a sample of a subject for diagnosis by steps (a-d); and f) comparing the detected band pattern
25 specific to the RNA obtained from a sample of subjects suffering from multiple myeloma from step (d) and the RNA obtained from a sample of the subject for diagnosis from step (f) to determine whether the patterns are the same or different and to diagnose thereby predisposition to multiple
30 myeloma if the patterns are the same.

In an embodiment, the size fractionation in step (b) is effected by a polyacrylamide or agarose gel. In an embodiment, the detectable marker is radioactive isotope,

enzyme, dye, biotin, a fluorescent label or a chemiluminescent label.

5 In an embodiment, multiple myeloma associated with the expression of a specific human MUM-1 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-1 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid
10 encoding MUM-1 protein.

15 In an embodiment, multiple myeloma associated with the expression of a specific human MUM-2 is diagnosed by the method for detecting a predisposition to multiple myeloma associated with the expression of a human MUM-12 protein in a DNA or RNA sample from a subject which comprises detecting in a sample from the subject a rearrangement of nucleic acid encoding MUM-2 protein.

20 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule. This invention provides an antisense oligonucleotide having a sequence
25 capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule.

30 This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA molecule

encoding a MUM protein. This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the RNA molecule encoding a MUM protein.

5 This invention provides a purified MUM protein. This invention provides a purified MUM-1 protein. This invention provides a purified human MUM-1 protein. This invention provides an antibody directed to a purified MUM-1 protein. This invention provides an antibody capable of specifically
10 recognizing MUM-1 protein. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

15 This invention provides a purified MUM-2 protein. This invention provides a purified human MUM-2 protein. This invention provides an antibody directed to a purified MUM-2 protein. This invention provides an antibody capable of specifically recognizing MUM-2 protein. In an embodiment, the antibody capable of specifically recognizing MUM-2
20 protein is a human MUM-2 protein.

In an embodiment, the antibody directed to a purified MUM-1 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein
25 is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-1 protein is a human MUM-1 protein.

30 In an embodiment, the antibody directed to a purified MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a monoclonal antibody. In an embodiment, the antibody capable of specifically recognizing MUM-2 protein is a human

MUM-2 protein.

5 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-1 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing
10 through a cell membrane.

15 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

20 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human MUM-1 protein and a pharmaceutically acceptable carrier
25 capable of passing through a cell membrane.

30 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding a human MUM-2 protein so as to prevent overexpression of the mRNA molecule effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing

through a cell membrane.

5 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a cDNA molecule encoding a MUM protein effective to prevent overexpression of a human MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

10 This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide having a sequence capable of specifically hybridizing to a genomic DNA molecule effective to prevent overexpression of a human
15 MUM-2 protein and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

20 This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

25

EXPERIMENTAL DETAILS

Materials and Methods

5 **Cell lines.** The following myeloma cell lines were used in
the present study: SK-MM-1, RPMI-8226, U266, EJM, XG-1,
XG-2, XG-4, XG-5, XG-6, XG-7, and XG-10. The RPMI-8226 cell
line was obtained through the American Type Culture
Collection (ATCC, Rockville, MD). SKMM-1 and U-266 cell
10 lines were gifts from Dr. A. N. Houghton and Dr. K. Nilsson,
respectively (18; 12). Characterization of these cell lines
were previously reported. Six XG cell lines were gifts from
Dr. B. Klein and were cultured in RPMI 1640 containing 10%
fetal calf serum (FCS), Sx10-smol/L 2-ME, and rIL-
15 6(1ng/mL) (13;19). Other myeloma cell lines used were all
IL-6 independent. The SK-MM-1 cell line was used to isolate
the chromosomal breakpoint carrying the 14q+ chromosome
without any information on the donor chromosome. XG-1,
XG-2, XG-6, XG-8 cell lines are reported to carry the
20 t(11;14)(q13;q32) translocation. XG-5 cells also share
both t(11;14) and t(8;14)(q24;q32).

Southern and Northern blot analyses. Southern blot analysis
was performed as previously described (21). Briefly, ten
25 micrograms of high molecular-weight DNA extracted from each
cell line was digested to completion with *Bam*HI and *Hind*III
restriction enzymes, size- fractionated on 0.7% agarose gel,
and transferred onto Duralose nitrocellulose membrane
(Stratagene) according to the manufacturer's instructions.
30 Blots were hybridized with a random-primed DNA probe and
washed at 60°C in 0.2 x SSC and 0.1 % SDS for 5 minutes.
Genomic probes used in this study were as follows; human IgH

J region JH probe (6.6kb *Bam*HI-*Hind*III fragment) was provided by Dr. J. V. Ravetch, human IgH C μ probe (1.3 kb *Eco*RI fragment) was provided by Dr. S.J. Korsmeyer. Human IgH region Cy2 probe was provided by Dr. C. Croce.

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Northern blot analysis was performed as described previously (21). Briefly, a 10 μ g aliquot of total RNA was loaded on each lane and probed with a 2.1H probe of the MUM1 gene (Figure 2A). GAPDH or β -actin probes were used as controls for amount of total RNA.

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Genomic library. High molecular-weight DNA of SK-MM-1 cell line was digested completely with *Bam*HI and partially with *Sau*3AI, and size-fractionated by using a low-melting point agarose gel. DNA ranging from 10kb to 23kb were purified and ligated into the *Bam*HI sites of λ -DASH II phage vector (Stratagene, La Jolla, CA). After packaging, 3×10^5 and 6×10^5 recombinant clones of the *Bam*HI digested library and partially digested library were screened with JH and C μ probes, respectively. To isolate the germline region of the 6p25 locus, a commercially available human placental library (Stratagene) was screened. Positive clones were mapped with restriction enzymes by partial digestion of the phage DNAs followed by probing with T7 and T3 primers labeled with T4 polynucleokinase and 32p- γ ATP.

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cDNA library. A phage library constructed by oligo-dT and random-priming normal human spleen RNA (Clontech) was screened by 2.1H probe (Figure 2A) to isolate initial MUM1 cDNA clones. After the first round of screening, positive clones were used as probes to walk to the 5' side using the same library. Positive clones were subcloned into

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pBluescript and analyzed for mapping and sequencing.

DNA sequencing. DNA sequences were determined by the dideoxy chain termination method and analyzed by an ABI(Applied Biosystems) autosequencer. Deletion mutants for sequencing were prepared using exonuclease III and mung bean nuclease. cDNA sequences were analyzed with the Genetics Computer Group (GCG) programs. Sequence homology searches were carried out through the BLAST E-mail server at the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD.

Fluorescence in situ hybridization (FISH). Metaphase chromosome from human lymphocytes were prepared. A biotin-labeled probe was prepared by nick-translation using Bio-16-dUTP. Conditions for hybridization and washing were described previously (22).

Experimental Results

IgH gene rearrangement of the SK-MM-I cell line. In *Bam*HI digestion, the JH probe detects two rearranged bands of the size of 12.0 kb and 9.7kb (Fig 1). The 9.7 kb band is comigrated with that probed with Cy2 probe, suggesting it to be a physiological rearrangement, although this cell line secretes only λ chain. One allele of the C μ locus is deleted and another is rearranged (6.5 kb) without being comigrated with rearranged bands of JH. Hybridization with a C α probe showed only the germline band (data not shown). These results suggested the possibility of the chromosomal breakpoint between JH and C μ locus. Hence, the 12.0 kb and the 6.5 kb bands detected by JH and C μ were considered to

represent unknown derivative chromosome and derivative 14 chromosome, respectively.

Molecular cloning of the t(6;14)(p25;q32) breakpoint.

A genomic library constructed with *Bam*HI complete digestion was screened with a JH probe to isolate the 12.0 kb *Bam*HI band. Another library constructed with *Sau*3AI partial digestion was screened with a C μ probe to isolate phage clones containing the 6.5 kb *Bam*HI fragment. Two phage clones, λ SKB-4a and λ SKS-3, considered to represent the unknown derivative and derivative 14 chromosomes respectively, were obtained (Fig 2A). A 0.7 kb *Bam*HI-*Hind*III probe (0.7B/H) of the λ SKS-3 was used to confirm the comigration with the rearranged 6.5 kb C μ band by Southern analysis (Fig 1). The chromosomal origin of the centromeric side of the λ SKB-4a and telomeric side of the λ SKS-3 were confirmed by hybridization to a somatic cell hybrid DNA panel with a 4.5 kb *Apa*I fragment(4.5A) and 2.1 kb *Hind*III(2.1H) probes. Both probes showed positive signals in hybrid cell DNA containing a human chromosome 6 (data not shown). These probes were also used to isolate the germline chromosome 6 region by screening the human placental genomic library. One of the phage clone DNA (λ MUM-3) was used as a probe for FISH analysis. It identified the localization of this region to be chromosome 6 short arm p25 (Fig 3). To investigate the precise breakpoint within the IgH gene, a 1.5 kb *Hind*III-*Eco*RI fragment of the λ SKS-3, containing the breakpoint on derivative 14 chromosome was sequenced. The breakpoint was confirmed to be just 3' to the switch μ (S μ) repetitive sequences (Fig 2B). Nucleotide sequencing of the region around the breakpoints of chromosome 6 and derivative 6

chromosome showed that the chromosomal translocation was reciprocal with minimum deletion of both the IgH and 6p25 sequences.

5 **Transcriptional unit in the vicinity of the 6p25 breakpoint.**

10 An attempt to find a functional transcriptional unit in the vicinity of the breakpoints was made. Although a 4.5A probe on derivative 6 chromosome could not detect any transcripts, a 2.1H probe on derivative 14 chromosome detected a single
15 6 kb transcript in the SK-MM-1 cell line. Accordingly, this gene was designated as *MUM1* (multiple myeloma oncogene 1). The same probe was used to study the expression of the *MUM1* gene in various hematopoietic cell lines. The 6 kb message
20 was expressed at high levels in most B cell lines and at low levels in peripheral T cell lines (Fig 4A). Cell lines derived from immature T cells, the myelomonocytic lineage, and erythroid lineage do not seem to express *MUM1*. In B cells, *MUM1* appears to be expressed throughout the development from the preB cell stage to the plasma cell stage (Fig 4B). However, some of the Burkitt's lymphoma
25 derived cell lines such as BJA-B did not express this gene (data not shown). The expression level of the *MUM1* transcript in myeloma cell lines was also examined (Fig 4C). The SK-MM-1 cell line showed a 7.5-fold overexpression when compared with the other three IL-6 independent cell lines, suggesting a deregulated expression of the translocated allele. It is of interest that the IL-6 dependent XG-4, XG-7, and XG-10 cell lines are also expressing at high
30 levels. Particularly, expression in the XG-7 cell line is 19.9 times the average of the aforementioned control cell lines.

MUMI cDNA cloning, sequencing, and homology search.

Human spleen cDNA library was initially screened with a 2.1H probe followed by three times walking to 5' side using cDNA probes. A 5.5kb cDNA, approximately corresponding to the size detected by Northern analysis was isolated. This cDNA contained a 1,353 base pair open reading frame (ORF) and a long 3' untranslated region (Fig 5A). The ORF encodes for a protein of 451 amino acids with a predicted molecular weight of 50 kD (Fig 5B). The putative ATG initiation codon at position 217 has G at the -3 position which corresponds to the Kozak consensus sequence (23). The ORF is preceded by two in-frame stop codons. A database search demonstrated a significant similarity between MUM-1 ORF and the interferon regulatory factor (IRF) family proteins. The NH₂-terminal of the MUM-1 ORF shares a high homology with all of the IRF family proteins which share a characteristic DNA binding motif consisting of the conserved 5 tryptophan residues (Fig 6A). The COOH-terminal also has a high homology with ICSBP (interferon consensus sequence binding protein) (21), ISGF3 γ (interferon-stimulated gene factor-3 gamma) (22), and IRF-3 protein (23) (Fig 6B), although it did not have any homologous regions with IRF-1 and IRF-2 protein. The highest similarity (95.1%) and identity (91.8%) were found with a possible mouse homolog, LSIRF (lymphoid specific interferon regulatory factor)/Pip (PU-1 cofactor protein-1) (24,25). A high similarity was found with ICSBP (63.98%), ISGF3 γ (55.8%), and IRF3 (50.1%) among the human IRF family protein members. A gene sequence encoding a nearly identical protein was recently deposited in GenBank. This gene, termed ICSAT (interferon consensus sequence binding protein in adult T-cell leukemia cell lines or activated T cells) is likely to be the same gene as MUM1

(26).

Breakpoints at MUM1 locus in multiple myeloma.

In order to analyze the exact location of the SK-MM-1
breakpoint at the 6p25 locus and to explore the frequency of
the MUM1 gene involvement in myeloma cases, we walked nearly
55kb in a human placental genomic phage library around the
MUM1 gene and determined the rough exon-intron structure as
shown in Figure 7 (Fig. 7). The SK-MM-1 breakpoint was
located 3' to the last exon, containing a poly A additional
signal, consistent with an unaltered size of the MUM1
transcript of this cell line in Northern analysis. Seven
repeat-free genomic probes shown in Figure 7 have been used
to investigate the rearrangement in Southern analyses of the
11 MM cell lines and 18 MM cases. One case (case 10)
displayed rearranged bands in BamHI and XbaI digests when
analyzed using a 0.9A probe located at 3' to the MUM1 gene.

**Cloning of the MUM2 locus from the U-266 multiple myeloma
cell line.**

Using an experimental strategy analogous to the one
described for the cloning of the MUM1 gene from the SK-MM-1
cell line, a second genetic locus altered in multiple
myeloma (MUM2) was identified by analyzing the U-266
multiple myeloma cell line. Briefly, Southern blot analysis
using BamH restriction digestion and various Ig probes showed
that U-266 DNA contained two rearranged fragments (shown by
arrowheads in Fig. 9) containing C α sequences and lacking J
sequences. These two fragments (der 14 and 14q32 in Fig. 9)
were cloned from a genomic library constructed from U-266
DNA along with a normal 14q 32 locus (14q32 germline in Fig.
9). In order to determine whether a gene was located in

proximity to the chromosomal breakpoints in der 14, the 2.5 BE restriction fragment (see Fig. 9), which was at the opposite side of the Ig Ca sequences, was used to probe a Northern blot carrying RNA from various MM cell lines. The results (Fig. 10) showed that a 1.9 kb mRNA was detectable in some of these cell lines including U-266. This result showed that a gene, called MUM2, normally not present within the Ig locus on chromosome 14q32, had been translocated in proximity of the Ig locus in U-266 cells. Since the Ig locus contains strong transcriptional regulatory elements, it is likely that the expression of this gene is deregulated in these cells. The structure of the MUM2 gene and its protein are currently under investigation. The 2.5 BE probe and other probes derived from the der 14 phage can be used to screen MM cases for MUM2 rearrangements as shown for MUM1 (Fig. 7).

Experimental Discussion

Using the experimental strategies used for the identification of the MUM1 and MUM2 genes in the SK-MM-1 and U-266 cell lines, respectively, it is possible to analyze most MM cases and isolate the corresponding genes. The scheme shown in Fig. 11 shows that the physiological IgH gene rearrangements (Fig. 11A) typically maintain linkage of C and J sequences and this linkage becomes detectable by using an appropriate restriction enzyme digestion (BamHI in the example in Fig. 11). Conversely, chromosomal translocations (14q+) affecting the IgH locus on 14q32 lead to breakage of the C-J linkage and the two sets of sequences appear on distinct restriction fragments. (Fig. 11B) Table 1 shows the application of this analysis to a panel of MM

cell lines and biopsies. The results show that at least 65% of cases show breakage of the C-J linkage within Ig J or switch regions. The restriction fragments containing either C or J sequences (R in Table 1) can be cloned as shown for the SK-MM-1 and U-266 cell lines and the genes flanking the chromosomal breakpoints can be used as probes to screen additional MM cases for similar rearrangements, whereas the sequence of the genes can be used to understand the consequences of these genetic lesions in multiple myeloma. Cloning of the chromosomal breakpoints and corresponding genes is currently ongoing for all of the MM cases shown in Table 1.

The method of analysis of 14q+ chromosomal translocations and identification of the genes altered in multiple myeloma of this invention will allow 1) the determination of chromosomal sequences involved in 14q+ translocations, the most important cytogenetic lesion associated with MM pathogenesis elucidation; 2) elucidation of specific gene lesions for MM; 3) a diagnostic method based on gene/DNA lesion and 4) a therapeutic approach aimed at counteracting the action of abnormal gene products.

Table 1. Summary of JH-C breakage analysis in MM cell lines and biopsies (cases). Rearrangement (R) involving physiologic Ig recombinations, i.e. retaining JH-C linkage are marked as R*; rearrangements lacking JH-C linkage, and therefore suggesting a 14q+ chromosomal breakpoint, are marked as R. The latter represents candidates for cloning an further analysis.

Cell Line/Case	sIg	JH	Cμ	Cα	Sγ3'	possible breakpoint locus
RPMI-8226	λ	D/D	D/D	G	<u>R</u> /G	Sγ
U-266	Eλ	R/D	D/D	<u>R</u> / <u>R</u> /G	G	Sα
EJM	Gλ	<u>R</u> /R*	D/D	G	R*/G	JH~Sμ
XG-1	Aκ	R*/D	D/D	R*	G	ND
XG-2	Gλ	R*/D	D/D	G	R*/G	ND
XG-4	Gκ	R*/ <u>R</u>	D/D	G	R*/G	JH~Sμ
XG-5	λ	<u>R</u> /D	D/D	G	G	JH~Sμ
XG-6	Gλ	R*/ <u>R</u>	D/D	G	R*/G	JH~Sμ
XG-7	Aκ	R/D	D/D	R/G	<u>R</u> /D	Sγ
XG-10	G	R*/ <u>R</u>	D/D	G	R*/G	JH~Sμ
SK-MM-1	κ	R*/ <u>R</u>	<u>R</u> /D	G	R*/G	JH~Sμ
CASE125		R*	G	G	R*	ND
CASE33		<u>R</u> /R*	G	G	<u>R</u> /R*	Sγ
CASE34		R*	G	R*	G	ND
CASE93		R*	G	R*	G	ND
CASE91		R*	R*	<u>R</u>	G	Sα
CASE128		R*	G	R*	G	ND

R*, comigrated bands with JH; R, target bands to isolate; ND, not determined

Possible breakage in switch regions:

Cell Lines	4/11 (36%)	
Cases	2/6 (33%)	Total 6/17 (35%)

Possible breakage in JH ~ switch regions:

Cell Lines	9/11 (82%)	
Cases	2/6 (33%)	Total 11/17 (65%)

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